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*Introduction*

Consideration of the mechanistic activities of BPA is an important component in its safety assessment. As with any toxicological evaluation this type of information generally plays a supportive role as scientists consider the biological plausibility of findings from animal or epidemiological studies that tend to feature more prominently in reaching conclusions on potential health concerns. In this context, consideration of BPA's mechanistic activities is especially important given that an increasing number of animal studies conducted over the past several years have reported health effects or implicated the involvement of cellular/physiological systems that extend beyond BPA's historical reputation as an environmental estrogen. It is not currently known the extent to which these non-nuclear estrogen receptor (ER) interactions account for some of the complexities encountered in the *in vivo* studies of BPA. However, they can complicate easy interpretation of findings when a response to BPA is compared to the response seen for the positive control estrogens, usually 17β-estradiol (E2), estradiol benzoate (EB), diethylstilbestrol (DES), or ethinyl estradiol (EE). This is especially true in "low dose" studies where BPA does not cause an effect predicted based on the positive control estrogen or elicits a response with a greater potency than predicted based on relative binding affinities or transcriptional activation of ERα or ERβ.

The conclusion that BPA should not be considered to act only as an estrogen, or even a SERM (selective estrogen receptor modulator), is supported by several lines of evidence, including *in vitro* or other types of mechanistic studies published in the peer-reviewed literature [reviewed in Wetherill et al. (2007) and Chapin et al. (2008)], results of EPA's high throughput screening program (ToxCast™) conducted through the Tox21 initiative[1], and comparisons of gene expression profiles for BPA with estrogens used as positive controls. It is worth noting that the assumptions that BPA glucuronide or BPA sulfate are not considered biologically active are

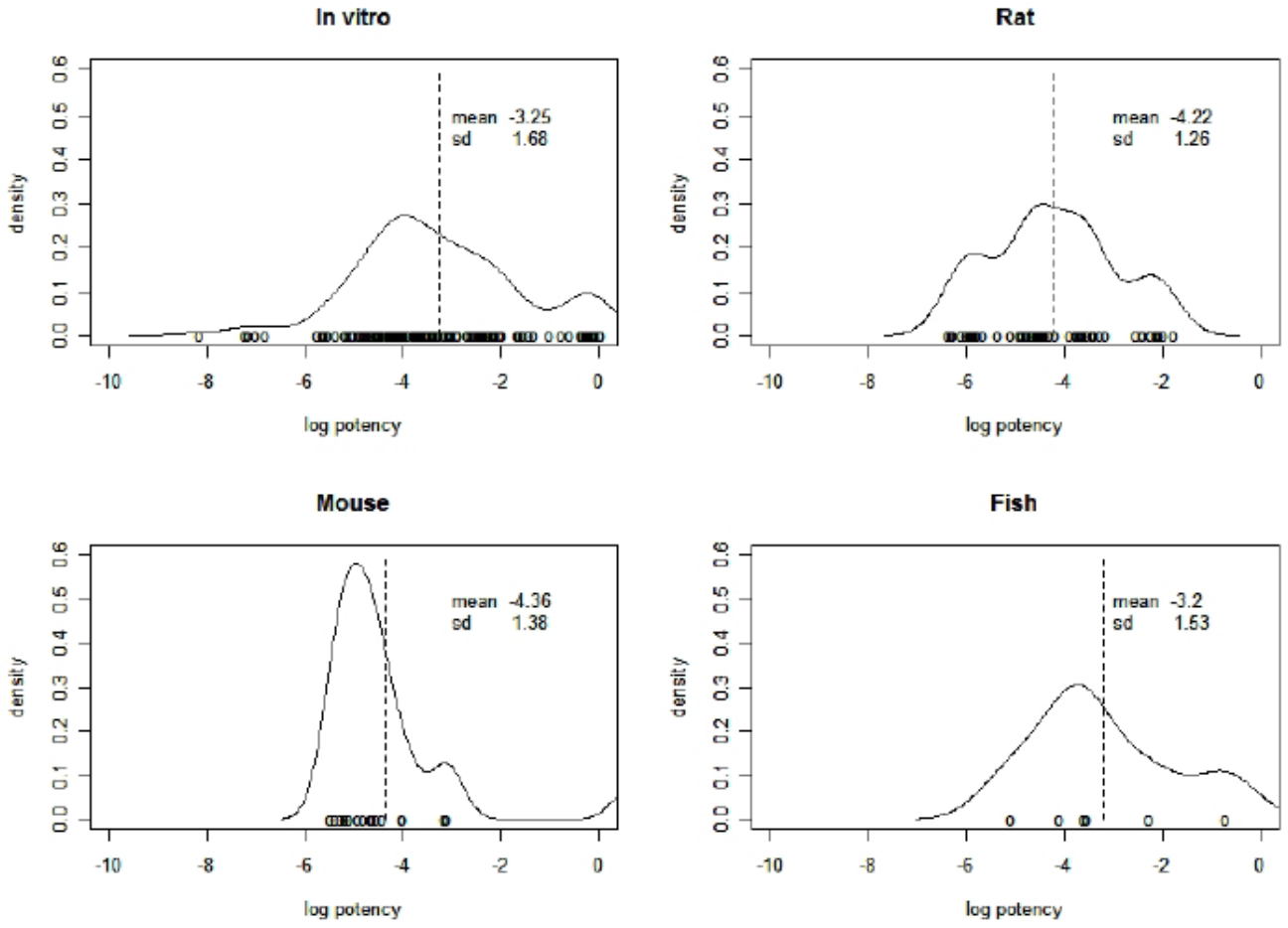
[1] Tox21 is a collaborative program between the EPA, NIEHS/NTP, NIH/NCGC, and FDA designed to research, develop, validate and translate innovative chemical testing methods that characterize toxicity pathways. Information on ToxCast and Tox21 is available at http://epa.gov/ncct/Tox21/ as well as overviews by Judson et al. Judson RS, Houck KA, et al. 2010. In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. Environmental health perspectives 118(4): 485-492. and Shukla et al. Shukla SJ, Huang R, et al. 2010. The future of toxicity testing: a focus on in vitro methods using a quantitative high-throughput screening platform. Drug Discovery Today In Press, Corrected Proof..

based on interactions with ERs (receptor binding) as other mechanistic activities have not been characterized (Chapin *et al.* 2008).

*Mechanistic Findings Based on Studies Published in the Peer-Reviewed Literature*

Many of the physiological effects of BPA have been described in the context of its ability to interact with classic ER (nuclear hormone receptor activity). The estrogenic activity of BPA was reviewed in detail by the NTP-CERHR Expert Panel on Bisphenol A (Chapin *et al.* 2008). The expert panel concluded that there is considerable variability in the results of individual studies with the estrogenic potency of BPA ranging over about 8 orders of magnitude, but similar means of 1,000-fold less potent than positive control estrogens *in vitro* and 1,000- to 10,000-fold less potent based on *in vivo* models in mice, rats, and fish (Figure 1).

**Figure 1. *In vitro* estrogenic potency (log10) in ERα and ERβ binding and transcriptional assays and estrogen-dependent cell proliferation assays and distributions of BPA and estrogen response *in vivo* in rats, mice, and fish**




From Figure 2 of Chapin et al. (2008). Each data point represents one BPA study in which BPA was compared to a reference estrogen in rats, mice, fish, or *in vitro* based on studies presented in Table 52 and Table 53 of the expert panel report.

In addition to nuclear hormone receptor activity, BPA also impacts cellular physiology through rapid signaling mechanisms to modify the activities of various intracellular signaling networks [reviewed in Wetherill et al. (2007)] . The rapid signaling effects of BPA are independent of nuclear hormone receptor activity and occur within minutes or seconds of exposure, initiated by membrane associated or intracellular receptor systems. Concentration response analysis in different experimental models has found that BPA is able to stimulate rapid signaling effects at concentrations in the pM to nM range. Maximal rapid signaling effects for BPA and 17β-estradiol are often observed at similar concentrations. The rapid signaling actions characterized by increased effects at lower concentrations, with decreased or loss of efficacy at higher concentrations that results in non-sigmodial (inverted-U shaped) concentration response curves [e.g. Nadal paper and (reviewed in Wetherill et al. (2007)]. It is considered likely that rapid estrogen-like signaling effects and "classical" nuclear hormone activity interact to coordinately impact physiological responses to estrogens.

Other *in vitro* studies have shown that BPA can interact with a number of other receptor systems. BPA has been reported to act as an anti-androgen with $IC_{50}$ values ranging from ~0.8 to 19.6 µM based on studies that assess the ability of BPA to reduce the binding of 5α-dihydrotestosterone (DHT) to the androgen receptor. The results vary depending on the model system and a study in HepG2 cells did not demonstrate any antiandrogenic activity [reviewed in Wetherill et al. (2007)]. The NTP recently conducted a reporter gene assay using a transient transfection of human full-length ERα or AR with a 3xERE-Luc or MMTV-Luc reporter, respectively into CV-1 cells. BPA was an androgen receptor antagonist with an $IC_{50}$ of 2.34 µM, a concentration that was about 10-fold higher than the $EC_{50}$ for ERα agonistic activity of 0.272 µ M. In this study, the antagonistic effect of BPA for the androgen receptor occurred at a concentration that was about 140-times higher compared to hydroxyflutamide ($IC_{50}$ of 0.0171 µM) and 7-times higher compared to the anti-androgen Casodex ($IC_{50}$ of 0.327 µM) while the agonist effect on ERα occurred at a concentration that was ~2,000-times higher compared to estradiol ($EC_{50}$ of 0.000126 µM) (personal communication with Dr. Tina Teng, September 9, 2010). These concentrations are lower than the activity of BPA for these receptors reported in Tox21 assays that use partial androgen and ERα receptors, where active concentrations, or $AC_{50}$ values, were reported of 17.9 to 73 µM for androgen receptor[2] and 0.64 to 1.723 µM for ERα.[3] Once bound to androgen receptor, the receptor-BPA complex may alter the ability of endogenous androgens to regulate androgen-dependent transcription [reviewed in Wetherill et al. (2007)].

There are also reports that BPA can interact with non-classic estrogen receptor systems at similar or lower concentrations than interactions with ERα and ERβ. BPA has a high binding affinity to estrogen-related receptor-γ (ERRγ), an orphan receptor that shares a sequence homology with ERα and ERβ but is not activated by estradiol (Coward et al. 2001; Greschik et al. 2004; Okada et al. 2008; Takayanagi et al. 2006). Takayanagi et al. (2006) reported that the $IC_{50}$ of BPA for ERRγ (13.1 nM) was ~80 to 100-times lower than the $IC_{50}$ values of BPA for ERα ($IC_{50}$

[2] Based on these assays: NCGC_AR_Antagonist and NVS_NR_hAR

[3] Based on these assays: ATG_ERE_CIS; NVS_NR_hER; ATG_ERa_Trans; and NCGE_ERalpha_Agonist. The NCGC also includes an assay for ERα antagonism, NCGC_ERalpha_Antagonist, and BPA had a $AC_{50}$ value of 17µM in that assay.

= 1040 nM) or ERβ ($IC_{50}$ = 1320 nM).[4] Also, BPA had a stronger binding affinity for ERRγ compared to DES ($IC_{50}$ = 13.1 nM versus 54.3 nM) but in contrast to DES or 4-hydroxy tamoxifen did not impact ERRγ's high basal transcriptional activity in report assays (Greschik *et al.* 2004; Takayanagi *et al.* 2006). ERRs can bind to both functional estrogen response elements (EREs) and ERR-response elements (ERREs), providing a mechanism through which ERRγ and ERα systems may cross-talk to mediate BPA's activities. In this model, a BPA-ERRγ complex may bind DNA together with E2-ERα via an ERE to create interference or cooperation to regulate overall estrogenic response in a particular cell type (Takayanagi, 2006 #453)). ERRγ can be found in the heart, brain, placenta, pancreas, kidney, prostate, brown adipose, breast and visceral adipose tissue, and muscle (Hugo et al. 2008; Kubo et al. 2009; Takayanagi *et al.* 2006; Takeda et al. 2009) and is implicated in regulating mitochondrial functions such as mitochondrial biogenesis, oxidative phosphorylation, adipocyte differentiation, and β-oxidation of fatty acids (Giguere 2008; Kubo *et al.* 2009) and behavior in zebrafish (Schletz Saili 2010).

BPA was reported to bind to G-protein-coupled receptor referred to as GPER (formerly GPR30) with an $IC_{50}$ of 0.630 µM, a binding affinity that was 2-3% of the $IC_{50}$ value for estradiol (0.0178 µM) (Thomas and Dong 2006). DES did not bind to this receptor at concentrations up to 10µM and ERα antagonist ICI182,780 and tamoxifen acted as agonist for GPER. While there is debate on whether GPER should be considered an estrogen receptor, it appears to contribute to some of the early actions of estrogen including rapid non-genomic signaling events that take place on the membrane (Olde and Leeb-Lundberg 2009). Understanding the physiological role of GPER is an area of active research and early studies suggest it may play a role in pancreatic islet cell function and glycemic control, bone growth, and immune and cardiovascular function (Liu et al. 2009; Maggiolini and Picard 2010; Martensson et al. 2009; Mizukami 2010; Nadal et al. 2009; Olde and Leeb-Lundberg 2009; Prossnitz and Barton 2009).

BPA has recently been described as an agonist for the glucocorticoid receptor (Prasanth et al. 2010; Sargis et al. 2010). Sargis et al. (2010) reported that 1µM of BPA (the only concentration tested) could increase luciferase expression in 3T3-L1 preadipocytes that were transfected with a glucocorticoid response element. Prasanth et al. (2010) used *in silico* molecular modeling and docking techniques to evaluate BPA's ability to bind to GR. BPA could be docked to the receptor and the nature of the interaction was described as similar to dexamethasone and cortisol and very different from the known GR antagonist RU-486. In ToxCast™, the $AC_{50}$ value for BPA binding to the glucocorticoid receptor (gene symbol NR3C1) was 10.5 µM.

Based on data from Tox21, BPA's activities for receptors involved in neural activities were apparent at higher concentrations than for ERα (ERS1), ranging from 10.8 µM for a serotonin receptor 6 (HTR6) to 27 – 38 µM for dopamine receptor 2 (DRD2), opioid receptor mu 1 (OPRM1), and opioid receptor-like 1 (Oprl1). The findings from ToxCast™ for thyroid hormone receptor contribute to an already inconsistent literature regarding BPAs ability to interact with these receptors. This literature was reviewed in Wetherill et al. (2007) and resulted in an overall

[4] An Attagene reporter gene assay for ERRγ in HepG2 cells is included in ToxCast and BPA was not considered active in this assay. However, the Attagene assay appears to have relatively low sensitivity, potentially due to low cellular expression (personal communication with Dr. Keith Houck, EPA, September 15, 2010).



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conclusion that the *in vitro* studies demonstrate that BPA binds to both the TRα and TRβ with relatively low affinity and BPA at physiological concentrations, (defined as ≤0.1µM) has a relatively minor influence on the thyroid hormone system. Also reviewed in Wetherill et al. (2007) were studies that assessed thyroid hormone parameters in other model systems, including amphibians. There were suggestions of molecular interactions at concentrations below the $IC_{50}$ for binding to thyroid receptor, suggesting that the nature of BPA's interaction with thyroid hormone signaling system is complex and unclear.

Another resource that is useful for considering the mechanism of action for BPA is the Comparative Toxicogenomics Database (CTD; http://ctd.mdibl.org/) a public resource being developed at the Mount Desert Island Biological Laboratory that includes manually curated data describing cross-species chemical–gene/protein interactions and chemical– and gene–disease relationships for many environmental chemicals. A November 15, 2010 search of CTD for the top 10 gene targets for BPA based on the peer-reviewed literature identified ESR1 (ERα), ESR2 (ERβ), PGR (progesterone receptor), CYP19A1, AR (androgen receptor), S100G (calbindin 3, or vitamin D-dependent calcium binding protein), LHB (luteinizing hormone beta polypeptide), MAPK1 (mitogen-activated protein kinase 1), NR4A1 (nuclear receptor subfamily 4, group A, member 1), and GH1 (growth hormone 1). However when considering the context of results from CTD, the rank order of gene targets associated with BPA does not necessarily reflect the degree of BPAs effect and in some cases may include findings that do not detect an interaction or effect (e.g THRA; see Xu et al., 2007 in CTD search results).

A large number of *in vitro* studies have looked at the "physiological" effects of BPA in a variety of cell systems, including endpoints such as cell proliferation, hormone release, and neuronal cell differentiation (reviewed in Wetherill et al. (2007)). These studies often report effects at lowest observed effect concentrations (LOEC) below $AC_{50}$ values for receptor binding. The types of *in vitro* studies summarized in Wetherill et al. (2007) include female reproductive tissue (LOECs from 0.0001-0.1 µM), breast cancer cells (LOECs from 0.0001-1 µM), male reproductive tissue (LOECs from 0.0001-150 µM), pancreatic/adipose tissue (LOECs from 0.0001-10 µM), pituitary (LOECs from 0.000001-1 µM), nervous system (LOECs from 0.0000001-2.5 µM), immune system (LOECs from 0.0001-10 µM), and embryonic development (LOECs from 0.1-1 µM). In addition, several recent studies have reported altered methylation patterns following treatment with BPA suggesting a role for epigenetic mechanisms (Avissar-Whiting et al. 2010; Bromer et al. 2010; Weng et al. 2010).

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*High Throughput Screening (HTS) Results from Tox21*

BPA was included in Phase 1 of ToxCast™, EPAs contribution to the high throughput screening (HTS) activities of Tox21. Phase I of ToxCast™ evaluated 309 chemicals, primarily pesticide actives, in 467 HTS assays that make use of 9 technology platforms. These platforms included both cell-free (biochemical) and cell-based measures in multiple human primary cells, human or rodent cell lines, and rat primary hepatocytes (Judson *et al.* 2010; Reif et al. 2010). Half-maximal activity concentrations ($AC_{50}$) are determined for each assay. A wide spectrum of biological targets or effects is covered, including cytotoxicity, cell growth, genotoxicity, enzymatic activity, receptor binding, ion channels, transcription factor activity and downstream consequences, gene induction, and high-content imaging of cells (Judson *et al.* 2010). Detailed information about each assay included in ToxCast™ is available from the ToxCast™ web site (http://www.epa.gov/ncct/ToxCast™).

Based on data from the 467 HTS assays, BPA was considered the 3rd most active of the 309 chemicals tested during Phase 1 using a prioritization scheme for endocrine activity developed by the National Center for Computational Toxicology (NCCT) (Reif *et al.* 2010). The NCCT visually conveys this information through ToxPi (Toxicological Priority Index) scores and graphics that include consideration of data from *in vitro* assays, chemical properties, and biological pathways (Figure 2). Endocrine profiles in Phase 1 were based on ToxCast™ assays for signaling pathways involving estrogen (6 assays), androgen (5 assays), and thyroid (5 assays) as well as other nuclear receptors (e.g., glucocorticoid receptor, peroxisome proliferator-activated receptor, pregnane-X receptor; 38 assays) and xenobiotic metabolizing enzymes that have potential relevance to endocrine signaling (cytochrome P450s, including aromatase; 36 assays). BPA's estrogen receptor (ESR1) agonist activities are a significant factor in its relative ranking. It's $AC_{50}$ or lowest effective concentration values from the 4 ESR1 assays included in Tox21 range from 0.64 to 1.72 µM.[5] However, BPA's relatively high overall endocrine score is also due to its activity relative to the androgen receptor, other nuclear receptors, XME/ADME (including the aromatase biochemical HTS assay), and the endocrine relevant pathway domain.

In terms of absolute "hit" rates, BPA had measurable activity in 101 assays, which made it one of the more broadly active of the 309 ToxCast™ chemicals. Many of these activities were identified at relatively high concentrations and the number of HTS target "hits" is reduced when using more stringent criteria based on $AC_{50}$ or LEC values below a preselected concentration threshold. Table 1 presents assay targets where BPA was considered active at concentrations of 10µM or lower. This table includes all assays where BPA was considered active. As noted above, the $AC_{50}$ values in this table reflect a range of assay technologies, cell types, and biological targets. The 3 main gene targets at $AC_{50}$ concentrations of <10µM are ESR1, xenobiotic sensing and metabolizing cytochrome P450s (CYPs) enzymes, as well as down-regulation of a number of inflammatory response genes in assays using human primary cell lines. At an AC50 of 0.16 µM, BPA interacted with a liver-specific organic anion transporter (SLCO1b1) of endogenous

[5] The NCGC also includes an assay for ERα antagonism, BPA had an $AC_{50}$ value of 17 µM in that assay.

compounds like bilirubin, 17-β glucuronosyl estradiol and leukotriene C4 that is also involved in the removal of drugs like statins from the blood into hepatocytes. As expected, the findings related to xenobiotic sensing and metabolizing are not unique to BPA and ~80% of the chemicals tested in ToxCast™ exhibit a similar response pattern (Judson *et al.* 2010). Indications of whole cell toxicity (e.g., cell cycle arrest, reduced hepatic cell viability, stress kinase) and genotoxicity were seen at high concentrations, generally with $AC_{50}$ values in excess of 100 µM. BPA also downregulated a number of genes involved in immune and inflammatory response, such as CXCL10, TGFB1, VCAM1, CCL2, and CD40, with $AC_{50}$ values of 4.44 µM for each. Another finding worth noting is that BPA exhibited binding to the glucocorticoid receptor (NR3C1) with an $AC_{50}$ of 10.5 µM. Activities for genes involved in neural activities were apparent at higher concentrations than for ERS1, ranging from 10.8 µM for serotonin receptor 6 (HTR6) to 27 – 38 µM for dopamine receptor 2 (DRD2), opioid receptor mu 1 (OPRM1), and opioid receptor-like 1 (Oprl1). BPA was not considered active at the concentrations tested for other receptors associated with neurological function, including muscarinic or nicotinic cholinergic receptors (CHRM1, CHRM2, CHRM3, CHRM4, CHRM5, CHRNA4), other dopaminergic receptors (DRD1, DRD4), other serotonin receptors (HTR2A, HTR2C, HTR3A, HTR5A, HTR7), and thyroid hormone receptor, α or β (THRA and THRB) (a full list of ToxCast™ gene assays is available at http://www.epa.gov/ncct/ToxCast™). An Attagene reporter gene assay for ERRγ in HepG2 cells is included in ToxCast™ and BPA was not considered active in this assay. However, the Attagene assay appears to have relatively low sensitivity, potentially due to low cellular expression (personal communication with Dr. Keith Houck, EPA, September 15, 2010).

Secondary analyses of gene targets included in ToxCast™ can also be conducted based on bioinformatics-based biological process/pathway databases developed by GO (Gene Ontology), KEGG (Kyoto Encyclopedia of Genes and Genomes), OMIM (Online Mendelian Inheritance in Man) and Ingenuity Systems. Table 2 summarizes the biological pathways associated with BPA by virtue of having at least 4 gene assays having an $AC_{50}$ of 10 µM or less. This information can provide some context for interpreting the ToxCast™ output but also comes with several caveats. First, the gene coverage of biological pathways within ToxCast™ varies and is quite limited for some pathways. The KEGG pathway for "Type II diabetes mellitus" is based on 44 genes but only 4 of these genes (TNF, INSR, MAPK3, and PRKCZ) are covered by assays within ToxCast™. The GO process "cardiac muscle cell proliferation" is covered by one gene (RXRA). Gene coverage is better for other biological pathways, 40 genes for the KEGG pathway "neuroactive ligand-receptor interaction" and 27 genes for the GO function "steroid hormone receptor activity." For these reasons, the biological process/pathway output based on the ToxCast™ data cannot necessarily be compared to conclusions reached by gene centric data of studies that look at thousands of genes. Second, diseases or biological pathways with very different names may be identified based on identical or similar ToxCast™ gene findings. In many cases for BPA its linkage to a biological pathway is being driven by genes for the ESR1 and cytochrome P450s (CYPs). For example, ESR1 gene activity links BPA to biological processes as divergent as OMIM phenotypes of "atherosclerosis" and "migraine susceptibility," and the GO process of "positive regulation of retinoic acid receptor signaling pathway." Similarly, BPA's association with the Ingenuity pathway "aryl hydrocarbon receptor signaling" was based on

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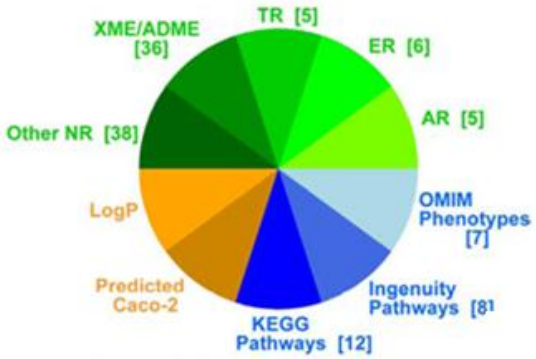
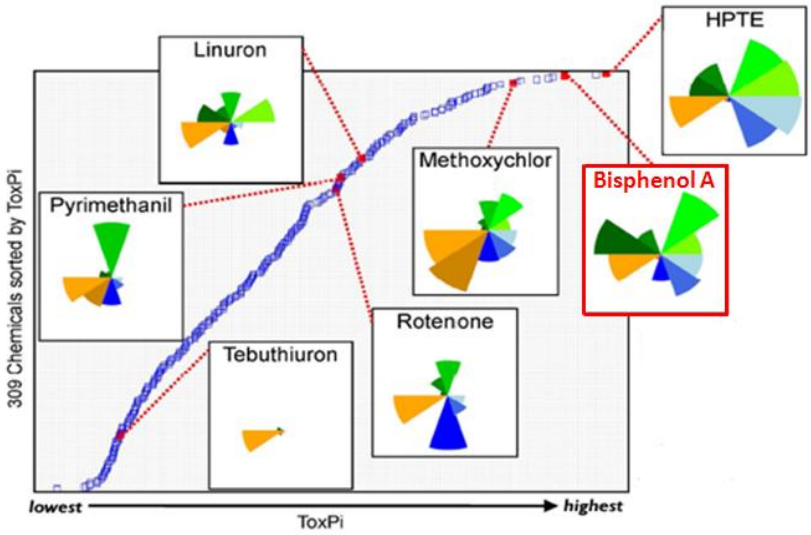
ESR1 rather than interactions with the aryl hydrocarbon receptor (AHR) that occurred at a concentration that was considered of little physiological relevance ($AC_{50}$ value of 32 µM). Third, BPA may also be associated with biological processes when it was not found to interact with the primary genes included in the process name. This occurred for the Ingenuity pathway for "PPARaRXRa activation"; BPA was associated with this pathway based on interactions with CYPs and TGFβ rather than PPARα or RXRα.

**Figure 2. Endocrine Screening ToxPi framework and reference chemicals along the distribution of sorted ToxPi scores for all 309 ToxCast™ Phase 1 chemicals (Reif et al. 2010)**

**A. ToxPi = f(*in vitro* assays + chemical properties + pathways)**




**B. BPA relative ranking in ToxCast™ Phase 1**




From Figure 4 of Reif et al. (2010) doi: 10.1289/ehp.1002180

## Appendix C: BPA Mechanisms of Action and other Biochemical/Molecular Interactions

(Updated December 27, 2010)

| Table 1. ToxCast™ HTS data for BPA (limited to activities at <10µM) | | | |
|---|---|---|---|
| **Official Full Name** | **Gene Symbol** | **Activation Target [Tox21 Assay Name]** | **AC50 (µM)[1]** |
| solute carrier organic anion transporter family, member 1B1 | SLCO1B1 | anion (drug) transporter [ToxCast™: CLZD_SLCO1B1_24] | 0.016 |
| nuclear receptor subfamily 1, group I, member 3 (Constitutive Androstane Receptor) | NR1I3 | CAR receptor [ToxCast™: NVS_NR_hCAR] | 0.111 |
| estrogen receptor 1 | ESR1 | nuclear receptor, estrogen receptor-α [ToxCast™: ATG_ERE_CIS; NVS_NR_hER; NVS_NR_bER; ATG_ERa_TRANS; NCGC_ERalpha_Agonist] | 0.640-1.723 |
| RAR-related orphan receptor gamma | RORγ | nuclear receptor, retinoic acid orphan receptor-γ | 0.891 |
| cytochrome P450, subfamily 2, polypeptide 11 | Cyp2c11 | Cyp2c11 is the most abundant member of the p450 xenobiotic-inducible superfamily in non-stimulated liver, metabolizes xenobiotics and testosterone. [ToxCast™: NVS_ADME_rCYP2C11] | 1.26 |
| CD69 molecule | CD69 | A member of the calcium dependent lectin superfamily of type II transmembrane receptors. Expression is induced upon activation of T lymphocytes; may play a role in proliferation and act to transmit signals in natural killer cells and platelets. [ToxCast™: BSK_SAg_CD69_up] | 1.48 |
| Mitochondrial toxicity | | [NCGC: 4 assays for mitochondrial toxicity] | 2.24-5.01, $EC_{50}$ |
| cytochrome P450, family 2, subfamily a, polypeptide 2 | Cyp2A2 | A hepatic steroid hydroxylase that is expressed exclusively in adult males, may play a role in drug metabolism.[ToxCast™: NVS_ADME_rCYP2A2] | 2.59 |
| cytochrome P450, family 3, subfamily A, polypeptide 4 | CYP3A4 | A member of the cytochrome P450 superfamily of enzymes which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids; involved in the metabolism of approximately half the drugs which are used today. [ToxCast™: CLZD_CYP3A4_48] | 3.53 |
| cytochrome P450, family 2, subfamily B, polypeptide 6 | Cyp2B6 | A member of the cytochrome P450 superfamily of enzymes which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. [ToxCast™: CLZD_CYP2B6_48; CLZD_CYP2B6_6] | 4.32-5.93 |
| chemokine (C-X-C motif) ligand 10 | CXCL10 | A chemokine of the CXC subfamily and ligand for the receptor CXCR3. Binding of this protein to CXCR3 results in pleiotropic effects, including stimulation of monocytes, natural killer and T-cell migration, and modulation of adhesion molecule expression. [ToxCast™: BSK_BE3C_IP10_down] | 4.44 |
| transforming growth factor, beta 1 | TGFB1 | A member of the transforming growth factor beta (TGFB) family of cytokines, which are multifunctional peptides that regulate proliferation, differentiation, adhesion, migration, and other functions in many cell types. Many cells have TGFB receptors, and the protein positively and negatively regulates many other growth factors. This gene is frequently upregulated in tumor cells, and mutations in this gene result in Camurati-Engelmann disease. [ToxCast™: BSK_BE3C_TGFb1_down] | 4.44 |
| vascular cell adhesion molecule 1 | VCAM1 | A member of the Ig superfamily and encodes a cell surface sialoglycoprotein expressed by cytokine-activated endothelium. This type I membrane protein mediates leukocyte-endothelial cell adhesion and signal transduction, and may play a role in the development of artherosclerosis and rheumatoid arthritis. [ToxCast™: BSK_hDFCGF_VCAM1_down] | 4.44 |
| chemokine (C-C motif) ligand 2 | CCL2 | Cytokines are a family of secreted proteins involved in immunoregulatory and inflammatory processes. | 4.44 |

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| **Table 1. ToxCast™ HTS data for BPA (limited to activities at <10µM)** | | | |
|---|---|---|---|
| **Official Full Name** | **Gene Symbol** | **Activation Target [Tox21 Assay Name]** | **AC50 (µM)[1]** |
| | | This cytokine displays chemotactic activity for monocytes and basophils but not for neutrophils or eosinophils. It has been implicated in the pathogenesis of diseases characterized by monocytic infiltrates, like psoriasis, rheumatoid arthritis and atherosclerosis. It binds to chemokine receptors CCR2 and CCR4. [ToxCast™: BSK_LPS_MPC1_down; ToxCast™: BSK_SAg_MCP1_down] | |
| CD40 molecule, TNF receptor superfamily member 5 | CD40 | A member of the TNF-receptor superfamily. This receptor has been found to be essential in mediating a broad variety of immune and inflammatory responses including T cell-dependent immunoglobulin class switching, memory B cell development, and germinal center formation. [ToxCast™: BSK_SAg_CD40_down] | 4.44 |
| cytochrome P450, family 2, subfamily C, polypeptide 9 | CYP2C9 | This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. [ToxCast™: NVS_ADME_hCYP2C9] | 5.25 |
| cytochrome P450, family 2, subfamily C, polypeptide 19 | CYP2C19 | This gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. Polymorphism within this gene is associated with variable ability to metabolize mephenytoin, known as the poor metabolizer and extensive metabolizer phenotypes. [ToxCast™: NVS_ADME_hCYP2C19_Activator] | 6.57 |
| cytochrome P450, family 2, subfamily a, polypeptide 1 | Cyp2A1 | Cyp2A1 has testosterone 7 alpha-hydroxylase activity and catalyzes 3,4 epoxidation of coumarin in coumarin metabolism. [ToxCast™: NVS_ADME_rCYP2A1] | 7.65 |
| cytochrome P450, family 2, subfamily C, polypeptide 18 | CYP2C18 | A member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum but its specific substrate has not yet been determined [ToxCast™: NVS_ADME_hCYP2C18] | 8.88 |
| cytochrome P450, subfamily 2, polypeptide 13 | Cyp2c13 | Cyp2c13 is a polymorphic cytochrome P450 isozyme with male specific expression. [ToxCast™: NVS_ADME_rCYP2C13] | 9.37 |
| cytochrome P450, family 1, subfamily A, polypeptide 1 | CYP1A1 | The CYP1A1 gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. The enzyme's endogenous substrate is unknown; however, it is able to metabolize some PAHs to carcinogenic intermediates. The gene has been associated with lung cancer risk [ToxCast™: NVS_ADME_hCYP1A1] | 9.62 |
| cytochrome P450, family 3, subfamily A, polypeptide 5 | CYP3A5 | A member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids and other lipids. This protein localizes to the endoplasmic reticulum and its expression is induced by glucocorticoids and some pharmacological agents. [ToxCast™: NVS_ADME_hCYP3A5] | 10.00 |

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## Appendix C: BPA Mechanisms of Action and other Biochemical/Molecular Interactions

(Updated December 27, 2010)

**Table 1. ToxCast™ HTS data for BPA (limited to activities at <10µM)**

| Official Full Name | Gene Symbol | Activation Target [Tox21 Assay Name] | AC50 (µM)[1] |
|---|---|---|---|
| nuclear receptor subfamily 3, group C, member 1 (glucocorticoid receptor) | NR3C1 | This gene encodes a receptor for glucocorticoids that can act as both a transcription factor and as a regulator of other transcription factors. The protein is typically found in the cytoplasm until it binds a ligand, which induces transport into the nucleus. [ToxCast™: NVS_NR_hGR] | 10.50 |
| 5-hydroxytryptamine (serotonin) receptor 6 | HTR6 | Serotonin 5-HT6 receptors are located primarily in the striatum, olfactory tubercles, nucleus accumbens and hippocampus. Functions of 5-HT6 receptors include modulation of cholinergic and dopaminergic neurotransmission, and they have a role in spatial learning and memory [ToxCast™: NVS_GPCR_h5HT6] | 10.80 |

[1]Values are expressed as $AC_{50}$ unless otherwise noted.

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**Table 2. Biological pathways associated with BPA based on gene assays included in Tox21**

| Biological Pathway | BPA "Active" Genes in Pathway (**GeneSymbol = BPA active in at least one assay for the gene with an $AC_{50}$ of ≤10uM;** GeneSymbol = BPA active in at least one assay, but with $AC_{50}$ of >10uM) |
|---|---|
| **OMIM Phenotype** | |
| Breast cancer | **ESR1** |
| Estrogen resistance | **ESR1** |
| Atherosclerosis | **ESR1** |
| HDL response to hormone replacement | **ESR1** |
| Migraine susceptibility | **ESR1** |
| Myocardial infarction susceptibility | **ESR1** |
| **GO function** | |
| Nitric oxide synthase regulator activity | **ESR1** AKT1 EGFR |
| Estrogen receptor activity | **ESR1** ESR2 |
| DNA binding | **ESR1, MTF2**, SERPINA3, ABL1, AEBP1, ALB, GLI1, MYB, MYC, NRF1, TCF7, SIRT2, SIRT3, SIRT1 |
| Protein binding | **NR3C1, HTR6, CCL2, TGFB1, VCAM1, NR1I3**, COL3A1, H2AFX, IL8, SERPINE1, POU2F1, PPARG, RARA, SELE, SLC6A2, NR1I2, MTF2 |
| Receptor activity | **ESR1, HTR6, NR1I3**, AR, CD38, DRD2, LDLR, OPRM1, PPARG, PTGER2 |
| Electron carrier | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CYP27B1 |
| Iron ion binding | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CYP27B1 |
| Metal ion binding | **ESR1, NR3C1, NR1I3, CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CYP27B1, PPARG, RARA, NR1I2, MTF2 |
| Zinc ion binding | **ESR1, NR3C1, NR1I3**, AR, MMP1, MMP9, PPARG, RARA, NR1I2, MTF2 |
| Protein N-terminus binding | **ESR1** |
| Transcription factor activity | **ESR1, NR3C1, NR1I3**, AHR, AR, JUN, NFE2L2, POU2F1, PPARG, RARA, NR1I2, CREB3 |
| Steroid hormone receptor activity | **ESR1, NR1I3** |
| Sequence specific DNA binding | **ESR1, NR3C1, NR1I3**, AR, JUN, NFE2L2, POU2F1, PPARG, RARA, NR1I2, CREB3 |
| Oxygen binding | **CYP1A1, CYP2C19, CYP2C18, CYP3A4, CYP3A5** |
| Heme binding | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CYP27B1 |
| Steroid binding | **ESR1, NR3C1**, AR |

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**Table 2. Biological pathways associated with BPA based on gene assays included in Tox21**

| Biological Pathway | BPA "Active" Genes in Pathway (**GeneSymbol = BPA active in at least one assay for the gene with an $AC_{50}$ of ≤10uM;** GeneSymbol = BPA active in at least one assay, but with $AC_{50}$ of >10uM) |
|---|---|
| Unspecific monooxygenase activity | **CYP1A1, CYP2B6, CYP2C18, CYP3A5**, CYP2J2 |
| Monooxygenase activity | **CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP27B1 |
| Oxidoreductase activity acting on paired donors with incorporation or reduction of molecular oxygen reduced flavin or flavoprotein as one donor and incorporation of one atom of oxygen | **CYP1A1, CYP2C19, CYP2C9, CYP3A4** |
| **GO process** | |
| Estrogen receptor signaling pathway | **ESR1**, RARA |
| Positive regulation of retinoic acid receptor signaling pathway | **ESR1** |
| Signal transduction | **ESR1, CD40, NR3C1, HTR6, NR1I3**, AHR, AR, CD38, DRD2, CXCL9, OPRM1, PLAUR, PPARG, PTGER2, RARA, NR1I2 |
| Inflammatory response | **CD40, CXCL10, CCL2, TGFB1**, IL8, CXCL9, SELE |
| Regulation of transcription, DNA dependent | **ESR1, NR3C1, NR1I3**, AHR, AR, JUN, POU2F1, PPARG, RARA, NR1I2, CREB3 |
| Oxidation reduction | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CYP27B1 |
| Transcription | **ESRI, NR1I3**, AR, PPARG, RARA |
| **GO component** | |
| Chromatin remodeling complex | **ESR1** |
| Extracellular region | **CD40, CXCL10, CCL2, TGFB1**, COL3A1, IL8, CXCL9, MMP1, MMP9, SERPINE1, PLAUR |
| Nucleus | **ESR1, NR3C1, TBGB1, NR1I3**, AHR, AR, H2AFX, JUN, NFE2L2, POU2F1, PPARG, RARA, NR1I2, CREB3, MTF2 |
| Nucleolus | **ERSR1, NR3C** |
| Cytoplasm | **ESR1, NR3C1, TGFB1**, AHR, AR, NFE2L2, SLC6A2 |
| Endoplasmic reticulum | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, OPRM1, CREB3 |
| Endoplasmic reticulum membrane | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2, CREB3 |
| Microsome | **CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Membrane | **CD40, CD69, CYP1A1, CYP2B6, CYP2C19, CYP2C9, CYP2C18, CYP3A4, CYP3A5, CCL2, VCAM1**, CYP2J2, CYP27B1, HLA-DRA, SELE, SLC6A2, CREB3 |
| Plasma membrane | **CD40, ESR1, HTR6, SLCO1B1**, CD38, DRD2, HLA-DRA, LDLR, OPRM1, SERPINE1, PLAUR, PTGER2, SELE |
| Integral to plasma membrane | **CD40, CD69, HTR6, SLCO1B1**, HLA-DRA, LDLR, OPRM1, PTGER2, SLC6A2 |
| Extracellular space | **CXC10, CCL2, TGFB1, VCAM1**, COL3A1, IL8, CXCL9, MMP9, SELE |

**Table 2. Biological pathways associated with BPA based on gene assays included in Tox21**

| Biological Pathway | BPA "Active" Genes in Pathway (**GeneSymbol = BPA active in at least one assay for the gene with an $AC_{50}$ of ≤10uM;** GeneSymbol = BPA active in at least one assay, but with $AC_{50}$ of >10uM) |
|---|---|
| **Ingenuity** | |
| Glucocorticoid receptor signaling | **ESR1, CCL2, TGFB1, VCAM1, NR3C1**, IL8, POU2F1, JUN, SERPINE1 , SELE, AR |
| Xenobiotic metabolism signaling | **CYP3A5, CYP2C9, CYP1A1, CYP2C19, CYP3A4, NR1I3, SLCO1B1**, NR1I2, NFE2L2**,** AHR |
| LPSIL-1-mediated inhibition of RXR function | **CYP3A5, CYP2C9, CYP2C19, CYP3A4, NR1I3,** NR1I2, RARA, JUN |
| ERKMAPK signaling | **ESR1**, CREB3, PPARG |
| PPARaRXRa activation | **CYP2C9, CYP2C18, CYP2C19, TGFB1**, JUN |
| Aryl hydrocarbon receptor signaling | **ESR1, CYP1A1, TGFB1**, RARA, AHR, JUN, NFE2L2 |
| Hepatic cholestasis | **ESR1, SLCO1B1**, NR1I2, RARA, JUN, IL8 |
| Hepatic fibrosis/hepatic stellate cell activation | **CCL2, TGFB1, CD40, VCAM1**, COL3A1, IL8, MMP9 |
| Estrogen receptor signaling | **ESR1, NR3C1** |
| **KEGG** | |
| Fatty acid metabolism (mus musculus) | **CYP1A1, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Tryptophan metabolism (mus musculus) | **CYP1A1, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Arachidonic acid metabolism | **CYP2C9, CYP2C19, CYP2C18, CYP2B6**, CYP2J2 |
| Arachidonic acid metabolism (mus musculus) | **CYP1A1, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Metabolism of xenobiotics by cytochrome P450 | **CYP1A1, CYO2B6, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5** |
| Metabolism of xenobiotics by cytochrome P450 (mus musculus) | **CYP1A1, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Linoleic acid metabolism | **CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Linoleic acid metabolism (mus musculus) | **CYP1A1, CYP2C9, CYP2C19, CYP2C18, CYP3A4, CYP3A5**, CYP2J2 |
| Cytokine receptor interaction | **CD40, CCL2, CXCL10, TGFB1**, CXCL9, IL8 |

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*Comparison of Gene Expression Profiles for BPA and Estrogenic Compounds*

Comparison of gene centric data between BPA and reference estrogens can be informative for determining the extent to which these estrogens should be considered appropriate positive controls in health effect studies of BPA. These comparisons for BPA with 17β-estradiol (E2) and two potent estrogenic compounds [17α ethinyl estradiol (EE), diethylstilbestrol (DES)], supports the notion that BPA should not be merely considered "estrogenic." Only a relatively small subset of genes is affected in common by BPA and these other compounds. This pattern is observed in studies conducted by multiple research groups and is based on comparative gene data collected from treated MCF7 breast cancer cells or Ishikawa endometrial cells, and using tissues collected from dosed animals. Collectively, the gene pattern findings support the conclusions reached in the review by Wetherill et al. (2007) that while BPA can act as an estrogen, its effects are cell specific and BPA is not simply a SERM (selective estrogen receptor modulator).

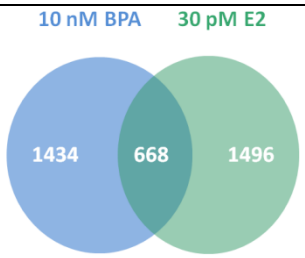
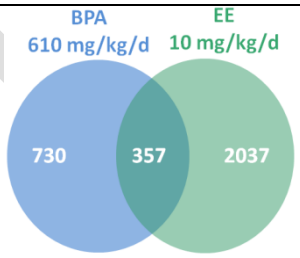
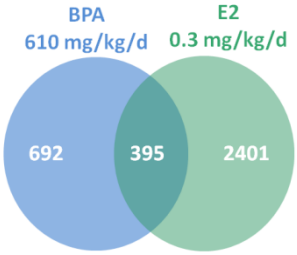
Figure 3 presents an analysis of genes affected by treatment with BPA or a positive control estrogen from several data sets included in NextBio Enterprise (Gollub ; Naciff et al. 2010; Shioda et al. 2006), a curated and correlated repository of experimental data from pharmaceutical and biotechnology companies, commercial and non-profit research organizations, and academic institutions (https://www.nextbio.com/b/nextbio.nb). The types of data included in NextBio include gene expression, proteomics, and microRNA expression for human, monkey, mouse, rat, fly, worm, and yeast. Inclusion of these data into NextBio permits secondary analyses, or "meta-analyses" of gene-centric data from the thousands of genes assessed in the original reports. The number of "overlapping" genes[6] affected by BPA and E2 or EE presented in Figure 3 range from ~7% to 36% of the total number of genes affected by BPA treatment alone.

Conclusions from this NextBio meta-analysis are generally consistent with the results presented in the published versions of these studies that used other strategies to determine the similarity in gene response to BPA and the positive control estrogen. For example, Naciff et al. (2010) looked at the response of 38,5000 human genes and expressed sequence tags (ESTs) in Ishikawa cells treated with 1 nM, 100 nM, 10 µM, or 100 µM BPA and sampled at 8, 24 and 48 hours after the start of treatment. The expression of 2794 genes was considered modified by BPA in a dose and time-dependent manner. A follow-up comparison of gene expression in Ishikawa cells treated with 100 µM BPA or 1 µM EE and sampled at 8, 24, and 48 hours after the start of treatment showed that the expression of 476 genes was affected by both BPA and EE. Among these 476 genes, the expression of 307 genes was altered in the same direction by these two chemicals, although the magnitude of the altered response was chemical specific. A similar pattern was reported in earlier studies from this same research group based on gene

[6] Overlapping, or "common, genes" include genes that are significantly affected (Welch test ($P<0.05$), fold change >1.2) by both the BPA and estrogen, regardless of the nature of the correlation (i.e., both increased or decreased by treatment or different direction of effect with the gene among the treatment groups).

expression patterns in reproductive tissues from fetal Sprague-Dawley rats that were transplacentally exposed to BPA at 5, 50, or 400 mg/kg/day; EE at 0.5, 1, or 10 µg/kg/day; or genistein at 0.1, 10, or 100 mg/kg/day via maternal treatment by subcutaneous injection (Naciff et al. 2005; Naciff et al. 2002). Approximately 7000 rat genes and 1740 ESTs were analyzed in the developing uterus and ovaries by Naciff et al. (2002) and the expression patterns for 397, 366, and 381 genes were considered altered (up- or down-regulated) by BPA, EE, and genistein, respectively. At the highest doses tested, the expression of 66 genes was consistently and significantly altered by all three compounds, although the magnitude of the response varied. A similar type of analysis at the highest doses using testicular/epididymal tissue from the fetuses showed that the expression of 37 of the 8740 genes assessed was modified in the same direction by at least 1.5-fold by BPA and EE (Naciff *et al.* 2005). The total number of genes that were uniquely altered at the highest dose tested compared to all lower doses in these treatment groups were 141 for EE and 67 for BPA.

**Figure 3. NextBio identified common genes between treatments with BPA and 17β-estradiol (E2) or 17α-ethinyl estradiol (EE)**

| | | | |
|---|---|---|---|
| MCF7 human breast cancer cells (48 hour treatment) Shioda et al. (2006) |  | Liver from adult male Sprague-Dawley rats treated for 5 days Gollub et al. (2008) |  |
| Ishikawa human endometrial cells with estrogen and progesterone receptors (24 hour treatment) Naciff et al. (2010) |  | Liver from adult male Sprague-Dawley rats treated for 5 days Gollub et al. (2008) |  |

* NextBio analyses conducted by Dr. Scott Auerbach, NTP Host Susceptibility Branch.

One of the studies included in Figure 3, by Shioda et al. (2006), compared the transcriptomal signature profiles of MCF7 cells following a 48-hour incubation with 30 pM E2, 10 nM BPA, 10 nM para-nonylphenol, 1 µM daidzein, 3 or 10 µM genistein, or 3 nM PPT [the selective ERα agonist tris (4-hydroxyphenyl)-4-propyl-1-pyrazole]. A mRNA levels of a similar number of genes were changed following treatment with BPA (2102 genes) and E2 (2164 genes); however, only 668, or ~30%, were affected in common. The gene expression response to 10 nM BPA was considered more similar to 10 nM para-nonylphenol compared to E2 or the other compounds included in the study (10 µM genistein > 1 µM daidzein > 3 nM PPT > 30 pM E2 > 3 µM genistein). Similarly, Boehme et al. (2009) found divergent gene expression patterns for BPA and DES in Ishikwa ER-plus and ER-minus cells following a 24-hour treatment (Table 3).

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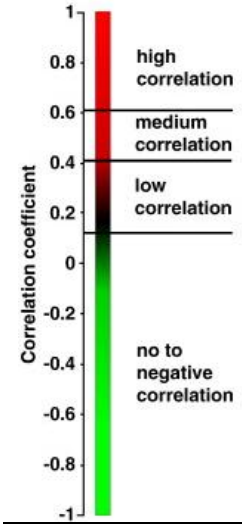
The top scoring biological pathways associated with the 10 nM BPA treatment[7] used by Shioda et al. (2006), as identified using NextBio, share some common features with the pathways identified by the U.S. Environmental Protection Agency (EPA) National Center for Computational Toxicology (NCCT) using in vitro screening data generated during Phase I of ToxCast™, namely metabolism of xenobiotics by cytochrome P450 and androgen/estrogen metabolism. A comparison of the Ingenuity pathway analysis of BPA and E2 expression data in MCF7 cells is presented in Table 4. One observation is that E2 seems to affect genes associated with immunological function more frequently or in magnitude than BPA.

Exposure to BPA in utero has been shown to affect the methylation status of several genes. (Dolinoy *et al*. 2007) found that giving a diet containing 50 mg BPA/kg of diet to the viable yellow agouti mouse (Avy) caused a shift in coat color from brown to yellow associated with a significant decrease in the methylation status of 9 CpG sites in a cryptic promoter region in the proximal end of an intracisternal A particle retrotransposon in the 5' end of the Agouti gene. (Bromer *et al.* 2010) reported that i.p. injections of pregnant CD1 mice with a BPA dose of 5 mg/kg bw per day on days 16-19 of gestation caused an increase in Hoxa 10 mRNA and protein expression in the reproductive tract of offspring. CpG methylation was decreased from 67 to 14% in the promoter region and from 71 to 3% in the intron.

**Table 3. Correlation of gene expression patterns between DES and BPA in Ishikawa cells (Boehme et al. 2009)**




| Treatment | Estrogen receptor status of Ishikawa cells | | Estrogenic response profile in Ishikawa "plus" cell line[1] | |
|---|---|---|---|---|
| | plus | minus | estrogenic | anti-estrogenic |
| DES (0.05nM ) | | | | |
| DES (1 nM) | | | 100% | 0% |
| BPA (2µM) | | | | |
| BPA (5µM) | | | 14% | 86% |

From Boehme et al. (2009)

[1] Percent estrogenic/anti-estrogenic response profile was calculated against the total number of significantly regulated genes for each compound.

[7] Top scoring BPA pathways from NextBio analysis of Shioda et al. (2006) data based on gene sets/canonical pathways from the Molecular Signatures Database (MSigDB): ribosome, irinotecan pathway, calcineurin Nf At signaling, pentose and glucuronate interconversions, metabolism of xenobiotics by cytochrome P450, androgen and estrogen metabolism, porphyrin and chlorophyll metabolism, starch and sucrose metabolism, taste transduction, G-protein coupled receptors pathway, cholesterol biosynthesis, stress pathway and glycolysis pathway

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**Table 4. Ingenuity pathway analysis of gene expression data from MCF7 cells treated for 48 hours with 10nM BPA or 30 pM E2 (based on data from Shioda et al. 2006)**

| BPA = E2 | BPA > E2 | BPA < E2 |
|---|---|---|
| actin cytoskeleton signaling | B cell receptor signaling | 14-3-3mediated signaling |
| aldosterone signaling in epithelial cells | cardiac hypertrophy signaling | allograft rejection signaling |
| axonal guidance signaling | colorectal cancer metastasis signaling | antigen presentation pathway |
| CCR3 signaling in eosinophils | ephrin receptor signaling | aryl hydrocarbon receptor signaling |
| CD27 signaling in lymphocytes | FAK signaling | ATM signaling |
| ceramide signaling | germ cell-sertoli cell junction signaling | autoimmune thyroid disease signaling |
| chronic myeloid leukemia signaling | GNRH signaling | biosynthesis of steroids |
| CXCR4 signaling | Huntingdon's disease signaling | breast cancer regulation by stathmin 1 |
| endometrial cancer signaling | IL-1 signaling | cell cycle: G2/M DNA damage checkpoint regulation |
| erythropoietin signaling | Notch signaling | cell cycle regulation by BTG family proteins |
| fMLP signaling in neutrophils | phospholipase C signaling | fMLP signaling in neutrophils |
| HER-2 signaling in breast cancer | PKCθ signaling in T lymphocytes | glioma signaling |
| HGF signaling | PPARα/RXRα activation | graft versus host disease signaling |
| HMGB1 signaling | RANK signaling is osteoclasts | granzyme B signaling |
| IGF-1 signaling | Reelin signaling in neurons | hereditary breast cancer signaling |
| insulin receptor signaling | regulation of actin-based motility by Rho | inositol metabolism |
| LPS-stimulated MAPK signaling | renal cell carcinoma signaling | mitotic roles of polo-like kinase |
| mTOR signaling | RhoA signaling | non-small cell lung cancer signaling |
| molecular mechanisms of cancer | role of NFAT in cardiac hypertrophy | ovarian cancer signaling |
| neuregulin signaling | SAPK/JNK signaling | pancreatic adenocarcinoma signaling |
| non-small cell lung cancer signaling | semaphorin signaling in neurons | pyruvate metabolism |
| NRF2-mediated oxidative stress response | | role of BRCA1 in DNA damage response |
| p53 signaling | | role of CHK proteins in cell cycle checkpoint control |
| PI3K/AKT signaling | | small cell lung cancer signaling |
| production of nitric oxide and reactive oxygen | | thrombopoietin signaling |
| prostate cancer signaling | | |
| Rac signaling | | |
| RAR activation | | |
| regulation of IL-2 expression in activated and anergic T lymphocytes | | |
| renin-angiotensin signaling | | |
| TR/RXR activation | | |
| type 1 diabetes mellitus signaling | | |
| VEGF signaling | | |

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